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Peripheral serotonin transporter DNA methylation is linked to increased salience network connectivity in female individuals with anorexia nervosa

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Abstract

Background

Epigenetic variation in the serotonin transporter gene (SLC6A4) has been shown to modulate the functioning of brain circuitry associated with the salience network (SN) and thereby may heighten the risk for mental illness. This study is the first to test this epigenetic-brain-behavior pathway in patients with anorexia nervosa (AN).

Methods

Resting state functional connectivity (rsFC) data and blood samples were obtained from 55 acutely underweight female AN patients and 55 age-matched female healthy controls (HC). Imaging data was decomposed using independent component analysis. Bisulfite pyrosequencing was employed to analyse blood DNA methylation within the promoter region of SLC6A4. SN rsFC patterns of the group × methylation interaction were interrogated.

Results

We identified a positive relationship between SLC6A4 methylation levels and rsFC between the dorsolateral prefrontal cortex and the SN in AN participants relative to HC. Increased SN rsFC was found to mediate the link between SLC6A4 methylation and eating disorder symptoms in AN. Findings of methylation-related rsFC alterations were confirmed for CpG-specific methylation at a locus with evidence of correspondence of methylation between brain and blood tissue.

Limitations

This study is cross-sectional in nature, the sample size is modest for the method and methylation levels were measured in peripheral tissue and cannot be generalized to brain tissue.

Conclusion

This study sheds light on the neuro-biological process of how epigenetic variation in the SLC6A4 gene may relate to rsFC in the SN that is linked to psychopathology in AN.

Introduction

The serotonin system has been associated with mood regulation, anxiety and the modulation of appetite[1], all aspects which nominate this neurotransmitter system as a potentialcandidate involved in the pathogenesis of anorexia nervosa (AN)[2]. AN is characterized by severe food restriction and affective symptoms, such as depressed mood and difficulties in emotion regulation[3], as well as a heightened prevalence of anxious and obsessive personality traits[4]. A number of studies suggest a reduced serotonergic tone during the acute undernourished state of the disorder, e.g. the serotonin metabolite 5-hydroxyindolacetic acid (5-HIAA) in cerebrospinal fluid[5] was found to be decreased, plasma prolactin response to serotonin agonists[6] were blunted and whole blood serotonin content as well as tryptophan levels[7] were reduced. In the weight-recovered state however, former AN participants may exhibit hyperserotonergic dysfunction[8]. The assumption of increased serotonin transmission being a trait-marker of the disorder is supported by decreased platelet MAO-B activity[9] and altered cerebral 5-HT_{1A} and 5-HT_{2A} receptor binding[10,11]. It has been hypothesized that serotonergic dysfunctions may drive body image distortions and self-starvation in AN[12].

However, associations between genetic polymorphisms of the serotonergic system, in particular the serotonin transporter SLC6A4, and AN could not be replicated[13]. Therefore an interest in the epigenome has gained momentum. Epigenetic processes such as DNA methylation may mediate environmental effects on gene expression without changing the DNA sequence. During DNA methylation, a methyl group is added to a cytosine residue, often adjacent to a guanine nucleotide. These so-called CpG-sites tend to cluster together in CpG-islands. In the promoter region of genes, CpG-islands are often demethylated, whereas the methylated state is commonly associated with reduced gene expression[14]. DNA methylation within the SLC6A4 promotor region appears to be of functional relevance as it has been found to predict lower SLC6A4 mRNA expression[15]. Furthermore, we have previously reported that DNA methylation at CpG site 13 (cg14692377) significantly correlates between blood and brain tissue obtained simultaneously from paired blood and temporal lobe biopsy samples[16]. This

CpG was also shown to correlate in paired pre-mortem blood and post-mortem brain samples. While SLC6A4 methylation has been reported to play a role in depressive disorders and schizophrenia[17], the only existing candidate-gene study in AN found no group differences in comparison to healthy controls (HC)[18]. A study using a methylome-wide approach also did not provide support for a link between SLC6A4 DNA methylation and a diagnosis of AN[19]. However, related phenotypes such as cortisol stress reactivity have been repeatedly associated with altered SLC6A4 methylation[20]. Expanding the scope by integrating epigenetic and neuroimaging data may allow us to test the complex interplay between genes, brain function and eating-disorder related abnormal behavior[21,22].

One promising neuroimaging technique is resting state functional connectivity (rsFC), which is defined as temporally correlated low frequency blood oxygen level-dependent (BOLD) signal fluctuation of distinct brain regions while the participant is at rest. This method is sensitive to changes in “communication” between spatially separated brain regions and hence has been argued to be more sensitive to the effects of genetic and environmental variation in heterogeneous phenotypes such as psychiatric disorders[23,24].

Recent evidence from rsFC studies points to a dysfunction in the salience network (SN) as a prominent feature of several mental disorders[23] including AN. The SN, comprised of the dorsal anterior cingulate cortex, the insular cortices and additional limbic structures such as the amygdala[23], is associated with detecting, filtering and integrating biologically relevant sources of salience such as emotional information, homeostatic regulation and reward value[25]. While some studies demonstrated aberrant functional within- and between network connectivity of the SN in AN, others found only indirect evidence for SN abnormalities in patients in the acutely underweight state[26,27]. Importantly, studies in healthy participants have observed that activity in key nodes of the SN including the amygdala[28] and insula[29] as well as amygdala connectivity[30] might depend on SLC6A4 methylation. Using a monozygotic twin-design, a recent study demonstrated an association between SLC6A4 methylation and brain responses

to negative stimuli in frontal-limbic regions overlapping the SN, that was independent of DNA sequence variations[31].

Based on the fact that previous studies in AN identified neural alterations in the SN, which has also been identified as neural correlate of SLC6A4 methylation, we assume that a serotonin-related epigenetic-brain-behavior pathway (discussed in detail in Palma-Gaudiel & Fananas[17]) may also be highly relevant for AN. The authors describe a neuro-biological pathway that is rooted in a SLC6A4 methylation mediated reduction of serotonin transporter expression. This reduction may lead to changes in the serotonergic tone, associated with altered reactivity and connectivity of SN-relevant brain regions and mental health problems.

Thus, in light of this proposed epigenetic-brain-behavior pathway for AN, the aim of this study is to examine the association between SLC6A4 methylation, SN rsFC and eating disorder (ED) symptoms in acutely ill AN patients compared to matched HC.

Methods and materials

Participants

The sample of the current study consisted of a total of 110 female volunteers: 55 acute AN participants according to DSM-5 (AN patients; 12-28 years old) and 55 pair-wise age matched female HC (12-28 years old). All AN patients were assessed within 96 hours after the beginning of a behaviorally-oriented nutritional rehabilitation program. Within the AN group, 53 of the patients were of the restrictive and two of the binge/purging subtype; seven had comorbid psychiatric disorders (four patients with depressive disorders including dysthymia, three with anxiety disorder and one with obsessive compulsive disorder). Six patients and 10 HC reported to smoke cigarettes currently or have been smoking in the past.

We applied several exclusion criteria for each group (see Supplemental Materials (SM) 1.1) – most importantly psychotropic medication other than selective serotonin reuptake inhibitors (SSRI) within four weeks prior to the study (n=1), binge eating, or diagnosis of bulimia nervosa,

substance abuse, neurologic or medical conditions. This study was approved by the Review Board of the TU Dresden and all participants (and if underage their guardians) gave written informed consent.

Clinical measures

To ascertain the absence or presence of a current ED, the expert form of the Structured Interview for Anorexia and Bulimia Nervosa for DSM-IV (SIAB-EX[32]) was conducted with all participants. Interviews were adapted to DSM-5 criteria (no amenorrhea criteria) and carried out by clinically experienced and trained research assistants under the supervision of the attending child and adolescent psychiatrist. IQ was estimated with a short version of the German adaption of the Wechsler Adult Intelligence Scale[33] or a short version of the German adaption of the Wechsler Intelligence Scale for Children[34] for participants aged 15 years or younger.

ED-specific psychopathology was assessed using the short version of the Eating Disorders Inventory (EDI-2)[35]. Depressive symptoms were examined using the German version of the Beck Depression Inventory (BDI II)[36]. Symptoms of anxiety were determined using the anxiety subscale of the Symptom Checklist 90 Revised (SCL-90-R)[37].

Blood DNA methylation sample processing and quality control

Quantitative peripheral DNA methylation at 15 CpG sites within the promoter-associated CpG island of SLC6A4 initially described by Philibert et al.[38] was performed by Varionostic GmbH (Ulm, Germany, http://www.varionostic.de). We focussed on 15 CpG sites within the amplicon 3 of a 799-bp region (originally CpG 43-57, referred to in the current study as CpG 1-15), as a prior study observed significant interindividual variation regarding methylation within this (but not in other) sub-region as well as a significant correlation with SN rsFC[30]. DNA methylation

within this sub-region has also been shown to correlate between blood and brain tissue[16]. Moreover, previous studies have argued that CpG-specific methylation rather than average methylation corresponds with mRNA expression[15,39]. To further investigate the correlation between peripheral, blood-based measures of SLC6A4 DNA methylation with those in post-mortem brain tissue, we queried BECon, an online database on cross-tissue DNA methylation[40]. Based on whole blood and post-mortem brain tissue from three regions (frontal, temporal and parietal) obtained from 16 individuals, cg14692377 showed moderate rank correlations between tissues (rho_{BA10} = 0.29 (frontal), rho_{BA20} = 0.36 (temporal), rho_{BA10} = 0.48 (parietal), indicating that in this specific case peripheral DNA methylation might indeed index brain-based methylation patterns. For analysis, genomic DNA was extracted from EDTA whole blood samples and bisulfite-treated using the EZ DNA Methylation Gold Kit (Zymo Research, Orange, CA). Subsequent pyrosequencing was performed on the Q24/ID System and percent DNA methylation at each CpG site was quantified using the PyroMark Q24 software (Qiagen). For a detailed protocol containing amplicon, sequencing as well as cross-CpG correlation and transcription factor binding site within the region, see SM 1.2. Pyrograms were visually inspected to individually verify DNA methylation estimates if they were at least three standard deviations away from the mean or if internal quality scores indicating a deviation from the expected pattern. Based on these criteria, we excluded data from one CpG (CpG 15). Mean missingness after exclusion was 0.58% across CpGs [range: 0 - 1.82%] and participants [range: 0 - 35.71%]. Missing values were subsequently imputed using predictive mean matching with default settings as implemented in the R mice package.

MRI-data acquisition and preprocessing

Data was acquired with a 3T Siemens Trio. The T1-weighted structural brain scans were acquired with a rapid acquisition gradient echo (MP-RAGE) sequence with the parameters as described in SM 1.3. An 8-minute resting fMRI scan was acquired by using a gradient-echo T2*-weighted echo planar imaging (EPI) using standard parameters (see SM 1.3). During fMRI, participants were instructed to lie still with closed eyes and without falling asleep.

Functional and structural images were processed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/) within the Nipype framework (http://nipy.sourceforge.net/nipype,) following standard procedures (SM 1.4).
We evaluated the quality of the fMRI data by manual inspection and using artifact detection tools (ART; SM 1.4).

Independent component analysis and identification of component of interest

To extract temporally coherent networks, that reflect well-defined circumscribed functional properties, a spatial group independent component analysis (ICA)[41] for all participants using the Group ICA fMRI Toolbox (GIFT) implemented in Matlab (http://mialab.mrn.org/software/gift) was conducted. Unlike seed-based approaches, this technique allows for an identification of a resting state network that is independent of neuroanatomical variability. After initial data reduction using principle component analysis, the fMRI data was decomposed into 24 maximally independent components by applying the infomax algorithm (SM 1.5). For each subject, component spatial maps were back-reconstructed using GICA and converted to *z*-values. As in our previous reports[42,43] components of interest were identified by spatial correlation with the relevant templates (salience, fronto-parietal and visual network) by Yeo et al.[44] Independent component number 9 (IC9) and number 13 (IC13) mapped onto the SN template defined by Yeo et al.[44] (SM 1.6).

Statistical models

We averaged DNA methylation levels across the 14 individual CpG sites after quality control and imputation to calculate a mean SLC6A4 methylation score ($methylation_{mean}$) for each participant[30]. While SLC6A4 $methylation_{mean}$ was our main measure of interest, we also investigated effects of CpG site 13 ($methylation_{CpG13}$), as there is evidence for small to moderate blood-brain correspondence of $methylation_{CpG13}$[16]. To identify the effects of

potentially confounding variables on DNA methylation we investigated associations with age (using Pearson's *r*).

Regarding rsFC, spatial maps of the components of each subject were entered into SPM8 to conduct the following analyses. First, to test for basic group differences, a two-sample t-test was performed for the two components identified as SN (step 1)[44]. Second, the main model was examined in components for which we had found a basic group difference at step 1. Second, rsFC in components which showed group difference in step 1 were regressed on group, SLC6A4 methylation$_{mean}$, their interaction and age as a covariate, with the interaction term being our main effect of interest (step 2). Third, based on previous findings as outlined above[15,16] we reran the main model described in step 2 using methylation$_{CpG13}$ instead of mean methylation level (step 3). Fourth, to investigate the specificity of the impact of DNA methylation on the rsFC of the SN, the group × methylation$_{mean}$ interaction was examined in a control analysis for the visual network (also identified by spatial correlation with a template[44]), a brain network which is thought to be somewhat less influenced by the serotonergic system[45] (step 4). Results of all models were masked using the aforementioned RSN templates and had to exceed $p<0.05$ family-wise error (FWE) to guard against type I errors. To test whether our main analysis (step 2) was influenced by cigarette consumption, we ran additional subanalyses excluding participants who reported to smoke currently or had been smoking in the past (AN=6; HC=10; also including occasional smoking) or were taking SSRIs (AN=1).

To develop a better understanding of the group × methylation$_{mean}$ interaction, we extracted β-values using the MarsBaR toolbox (http://marsbar.sourceforge.net/) at a threshold of $p<0.001$ (uncorrected; to account for greater anatomical variability) and subjected those to further testing using SPSS statistical software version 21.0 (SPSS, Chicago, Illinois). The associations with the physiological and psychometric parameters BMI-standard deviation score (BMI-SDS)[46], EDI-2, BDI-II and the anxiety subscale of SCL-90-R were examined using Pearson's

r for each diagnostic group separately. Additionally, an analysis of the association with the core-scales of the EDI-2 (, drive for thinness and body dissatisfaction) is included in SM 2.4

In order to probe the proposed serotonin-related epigenetic-brain-behavior pathway, we conducted a mediation analysis using PROCESS package implemented in SPSS. To this end, we tested whether the link between alterations in SLC6A4 $methylation_{mean}$ and elevated psychopathology (EDI-2 total, BDI-II and SCL-90-R anxiety) is mediated by altered rsFC in the SN (indirect effect) in AN participants using the bootstrapping procedure on 20000 samples to compute the lower and upper level of the 95% confidence interval.

Results

Participants

As shown in Table 1, there were no significant group differences in age. As expected, AN patients had a lower BMI-SDS as well as a higher EDI-2 total score, BDI-II score and SCL-90-R anxiety level than HC. AN patients did not differ from HC in *SLC6A4* $methylation_{mean}$ or $methylation_{CpG13}$ (Table 1). SLC6A4 $methylation_{mean}$ was significantly associated with age (r_p=0.22; p=0.021), thus we included age as a covariate in our main statistical models.

Moreover, we investigated whether *SLC6A4* $methylation_{CpG13}$ is dependent on genetic variation. Based on publically available data, we could not identify underlying methylation quantitative trait loci in blood (mqtldb.org) or human brain tissue (epigenetics.essex.ac.uk.mQTL; SM 2.6).

Group comparison of independent components and association with methylation

A two-sample t-test of the two SN components (IC 13 and IC9) revealed increased rsFC within the left (*t*=4.68; *p*=0.006; *k=15* (FWE)) and right (*t*=4.88; *p*=0.014; *k=7* (FWE)) posterior insula

and IC9 for AN in comparison to HC (step 1; Figure 1). No group difference was identified for IC13. In step 2, we identified a group × $methylation_{mean}$ interaction in the right dorsolateral prefrontal cortex (dlPFC) for IC9, controlling for age (t=4.49; p=0.016; k=*6* [32; 40; 34] (FWE); Figure 2, panel A). Detailed analysis of this interaction revealed that SLC6A4 $methylation_{mean}$ correlated positively with rsFC between this region and other areas of IC9 in AN patients (r=0.425; p=0.002), while this correlation was negative in HC (r=-0.408; p=0.002) (Figure 2, panel B). In step 3, we tested specifically the group × $methylation_{CpG13}$ interaction and observed an identical neural pattern in the right dlPFC (t=4.73; p=0.018; k=*5* [34; 38; 30] (FWE), see SM 2.1).

Control analysis in the visual network revealed no group x $methylation_{mean}$ interaction, suggesting that the finding described above was specific to the SN (step 4). Exclusion of participants with current or past cigarette consumption confirmed our initial results in the SN (t=5.09; p=0.007; k=*13* [30; 40; 32] (FWE)). Excluding the participant who took SSRI also had no influence on the results (t=4.46; p=0.018; k=*5* [32, 40; 34] (FWE)).

Moreover, increased rsFC between the significant cluster in the dlPFC, for which we reported the group × $methylation_{mean}$ interaction, and other regions of the SN was associated with ED psychopathology, indicated by a correlation with the EDI-2 total score, but not with BMI-SDS, BDI-II or SCL-90-R anxiety, within the patient group (r_p=0.349; p=0.010; withstands Bonferroni-correction for four tests). Mediation analysis showed that the effect of SCL6A4 $methylation_{mean}$ on EDI-2 total, but not on BDI-II or on SCL-90-R anxiety, was mediated by alterations in rsFC of the SN in AN participants (see Table 2 and SM 2.5).

Discussion

The present study is the first to investigate a potential epigenetic-brain-behavior pathway in AN by examining the association of SLC6A4 methylation and resting state rsFC of the SN in patients acutely ill with this disorder and HC. On a group level, we observed increased rsFC in the SN at the posterior insula in patients compared to controls, but – in agreement with

previous reports[18,47] – no difference in SLC6A4 methylation levels. However, we did identify a positive association between methylation levels and rsFC between the dlPFC and other regions of the SN in AN participants, which was also associated with elevated ED symptoms. In fact, alterations in SN rsFC were found to mediate the link between SLC6A4 methylation changes and ED but not depressive or anxiety-related psychopathology. These results may suggest that this methylation-related rsFC pattern is specific for ED symptoms. However, to confirm this notion further research including other clinical groups is needed. In HC, increased SLC6A4 methylation levels were associated with reduced rsFC in this brain region. Importantly, these associations could be demonstrated for mean methylation levels as well as for CpG-specific methylation (*SLC6A4* methylation$_{CpG13}$) at a locus with previous evidence for a correspondence of methylation levels across blood and brain tissue[16]. Of note, no association between methylation and rsFC was detected in an *a priori* chosen negative control network – the visual network, indicating that the found association may be specific for the SN.

Alterations in the SN have been described to underlie abnormal sensitivity to salient external or internal events with severe consequences for cognition and behavior, as described across several mental disorders[48] including AN[26]. The posterior insula, the location of the general group difference in the SN, has been associated with pain and sensorimotor representation and is thought to function as a major interoceptive region[49]. In line with this, we have previously demonstrated altered functional network characteristics in the posterior insula in AN[50,51], which may reflect inefficient information transfer to and from this brain region. Functional alterations in the posterior insula have also repeatedly been shown in AN using task-based fMRI[52]. Taken together, dysfunctions in the insular cortices relate to impaired integration of visuospatial, homeostatic and interoceptive signals, which may account for some of symptoms in AN, such as body image distortions, elevated pain threshold and severe self-starvation[53].

Our finding of methylation-related SN rsFC alterations, which was also associated with ED severity in AN, fits well with a previously reported notion of a clinically relevant brain-mediated association between SLC6A4 methylation and illness-related behavior[28,30,54,55]. In detail,

previous neuroimaging studies in health and disease (for review see Palma-Gudiel & Fañanás[17]) − as well as the here reported findings in AN − suggest that increased SLC6A4 methylation may link to heightened activity and connectivity in SN-related brain regions in individuals with severe mental health conditions. In contrast to AN, we observed a negative (rather than positive) association between SLC6A4 methylation and rsFC in the SN in HC. Similarly, Frodl et al.[29] demonstrated a negative association between methylation and neural reactivity in the posterior insula, pons and temporal pole when shifting attention away from emotional stimuli in a sample including HC and patients with major depressive disorder. In sum, the direction and exact localization of the association between SLC6A4 methylation and brain functioning is still somewhat unclear and difficult to compare due to different employed fMRI methods (resting state [seed-based[30] versus ICA approach) or task fMRI] and contrast conditions in task fMRI.

Interestingly, while previous studies investigating SLC6A4 methylation-related brain regions focused primarily on ventral-limbic parts of the brain such as the amygdala, we found a methylation-related increase in rsFC between the dlPFC and other regions of the SN in AN. In order to further probe the specificity of this finding regarding the SN, we also tested the fronto-parietal network (network with the dlPFC as a central hub) as part of an additional follow-up analysis (SM 2.3). Similar to the visual network (negative control network), we could not detect a significant association between methylation and rsFC of the fronto-parietal network. Given that the methylation-related increase of rsFC was present in a section of the dlPFC belonging to the SN, but not for subregions of the dlPFC, which are part of the fronto-parietal network, we suggest that the associations with SLC6A4 methylation may be specific to the fronto-limbic circuit. This circuit is a component of the SN and part of the serotonin pathway, i.e. it is strongly innervated by serotonergic neurons and rich in various serotonin receptors[56]. With regard to processing emotions and saliency – a major function of the SN – the dlPFC (and its coupling with limbic regions) is also engaged during bottom-up appraisal of emotions[57] and during emotion regulation via cognitive strategies[58]. Modulation of frontal-limbic circuits via the serotonergic system has previously been shown by studies focusing on the 5-HTTLPR. This

polymorphism is thought to modulate the association between lateral prefrontal cortex structure[59] and biased attention to emotional stimuli[60]. Moreover, 5-HTTLPR was observed to be linked to cognitive symptoms present in mental disorder such as obsessive-compulsive disorder[61] and AN[62]. Based on this, a speculative interpretation of our findings could be that a methylation-related increased involvement of the dlPFC in the SN is related to elevated cognitive or self control – a much debated characteristic of AN[2]. This tentative interpretation of the findings dovetails with the notion of AN as a model-disorder of increased cognitive control, which may be particularly reflected in the tendency of patients to respond in a strategic rather than hedonic manner to salient stimuli[63,64]. Interestingly, in a recent study we discovered a sustained lateral prefrontal cortex responsivity in AN participants when food-related, social but also neutral stimuli were presented[64]. We speculated that this may reflect an inability of AN patients to disengage cognitive control. In light of this, the current findings of a SCL6A4-associated increased rsFC between dlPFC and the rest of the SN – although correlational in nature - may be interpreted as a tendency of AN patients to 'stay in control', which could potentially be a neural downstream effect of elevated SLC6A4 methylation, leading to unwanted effects such as increased ED symptoms.

Limitations

The interpretation of the present study rests upon the following limitations. First, the cross-sectional nature of the study does not allow determining a causal link between epigenetic variation, brain network characteristics and the emergence of ED symptoms or whether the observed alterations are an effect of undernutrition. In order to shed light on temporal relationships, longitudinal study designs are needed. Second, methylation measures were assessed in peripheral tissue, which cannot be generalized to neural tissue. However, previous work could demonstrate a substantial association between the methylation-level in blood and brain for *SLC6A4* methylation$_{CpG13}$[16,65], for which we could confirm our initial findings. Furthermore, while methylation at this site was somewhat low , which could add noise due to technical variation, the methylation levels in our study are in line with previously reported

levels[66]. Third, the present study only focused on epigenetic variation but failed to incorporate genetic variability and other influencing factors such as stress and childhood adversity. While the contribution of both epigenetic and genetic factors might be relevant to predict the expression of SLC6A4[17], *SLC6A4* methylation$_{CpG13}$ does not seem to be under strong genetic control. Fourth, rates of comorbidity were lower than previously reported [67] which could be due to the fact that no structured interview other than the SIAB-EX was used (see SM 1.1).

In conclusion, our findings suggest, if replicated, that a serotonin-related epigenetic-brain-behavior pathway is also relevant for AN. It sheds light on the neuro-biological process of how epigenetic variation of the SLC6A4 gene may associate with rsFC in the SN that is specifically linked to ED symptoms. Such knowledge may spur future research on the potential of epigenetic markers to assess risk for disordered eating and pave the way for potential novel pharmacological and behavioral treatment strategies that modulate methylation levels of specific genes[68,69].

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Table 1: Demographic and clinical characteristics; If not otherwise indicated mean and standard deviations are reported; BMI-SDS=body mass index standard deviation score, EDI-2=eating disorder inventory 2; BDI-II=Beck's depression inventory 2; SCL-90-R=Symptom Checklist 90 Revised; AN=patients acutely ill with anorexia nervosa; HC=healthy controls.

Table 2: Mediation analysis between SCL6A4 methylation$_{mean}$ and the outcome variables EDI-2 total and BDI-II with rsFC of the SN as mediator; LLCI=lower level of the confidence interval; ULCI=upper level of the confidence interval; EDI-2=eating disorder inventory 2; BDI-II=Beck's depression inventory 2.

Figure 1: Two-sample t-test of the salience network; A two-sample t-test within the IC9 revealed an increased functional connectivity between the left (t=4.68; p=0.006 (FWE)) and right (t=4.88; p=0.014 (FWE)) posterior insula and the rest of the component for AN in comparison to HC.

Figure 2: Group × SLC6A4 methylation$_{mean}$ interaction; A) Results of the group × SLC6A4 methylation$_{mean}$ interaction analysis in the SN at the dorsolateral prefrontal cortex cluster (dlPFC) (for illustrative purposes shown at p<0.001, uncorrected). Applied SN mask [44] is depicted in transparent grey. Color bar represents t-values. B) Plot of association of the extracted β-values of the dlPFC cluster and mean SLC6A4 methylation (in %) separately for AN and HC. AN=patients acutely ill with anorexia nervosa; HC=healthy controls.